AllBio V5 Zero Cloning Kit

Please read the user manual carefully before use.

Cat. No. ABTGMBV501

Storage

AllBio T1 Phage Resistant Chemically Competent Cell at -70°C for six months; others at -20°C for nine months

Description

AllBio V5 Zero Cloning Vector contains a suicide gene. Ligation of PCR fragment disrupts the expression of the gene. Cells that contain non-recombinant vector are killed upon plating. Therefore, blue/white screening is not required.

- 5 minutes fast ligation of Taq-amplified PCR products.
- High cloning efficiency. Positive clones up to 100%.
- No blue/white selection needed.
- Suitable for short and large fragment cloning.
- Kanamycin and Ampicillin resistance genes for selection.
- M13 forward primer and M13 reverse primer for sequencing.
- T3 promoter and T7 promoter for in vitro transcription.
- AllBio T1 phage resistant chemically competent cell, high transformation efficiency (>109 cfu/μg pUC19 DNA) and fast growing.

Kit Contents

Component	10 rxns
AllBio V5 Zero Cloning Vector (10 ng/μl)	10 μl
Control Template (5 ng/μl)	2.5 μl
Control Primers (10 μM)	2.5 μl
M13 Forward Primer (10 μM)	25 μl
M13 Reverse Primer (10 μM)	25 μl
AllBio T1 Phage Resistant Chemically Competent Cell (Option) -70°C Cat. ABTGCD501-10	5x100 μl

Preparation of PCR Products

1. Primer requirement: primer cannot be phosphorylated
2. Enzyme choice: Taq DNA polymerases
3. Reaction conditions: in order to ensure the integrity of amplification products, 5-10 minutes of post-extension step is required. After amplification reaction, use agarose gel electrophoresis to verify the quality and quantity of PCR product

Setting Up the Cloning Reaction System

Add following components into a microcentrifuge tube.

PCR products----------0.5-4 μl (can be increased or reduced based on PCR product yeild, not more than 4 μl)

AllBio V5 Zero Cloning Vector------1 μl

Gently mix well, incubate at room temperature (20°C-37°C) for 5 minutes, and then place the tube on the ice.

1. Optimal amount of insert
   Molar ratio of vector to insert = 1:7 (1 kb, ~20 ng; 2 kb, ~40 ng)
2. Optimal volume of vector: 1 μl (10 ng)
3. Optimal reaction volume: 3~5 μl
4. Optimal incubation time

(1) 0.1~1 kb (including 1 kb): 5~10 minutes
(2) 1~2 kb (including 2 kb): 10~15 minutes
(3) 2~3 kb (including 3 kb): 15~20 minutes
(4) ≥3 kb: 20~30 minutes

Use the maximum incubation time if the insert is gel purified.

AllBio V5 Zero Cloning Kit

5. Optimal incubation temperature: for most PCR inserts, the optimal temperature is about 25°C; for some PCR inserts, optimal results can be achieved with higher temperature (up to 37°C).

Transformation

1. Add 5 µl of ligation products into 50 µl of AllBio T1 Chemically Competent Cell (ligation product should be added immediately after thawing the cells on ice) and mix by tapping gently. Incubate on ice for 20-30 minutes.
2. Heat shock at 42°C water bath for exactly 30 seconds, and place on ice for 2 minutes.
3. Add 250 µl of room temperature SOC or LB medium. Incubate at 37°C for 1 hour. at 200 rpm
4. Spread 200 µl transformants on the plate and incubate overnight (to obtain more colonies, centrifuge cells at 4000 rpm for 1 minute, discard a portion of supernatant and keep 100-150 µl of it. Gently tapping to suspend the cells, plate all the cells and incubate overnight).

Identification of Positive Clones and Sequencing

Identify Positive Clones by PCR

1. Pick several colonies and place them in 10 µl sterile water, mix by vortexing.
2. Add 1 µl mixture into 25 µl reaction volume. Identify positive clones with M13 forward primer and reverse primer.
3. PCR reaction conditions

94°C	10min	
94°C	30 sec	30 cycles
50-60°C	30 sec	
72°C	x min*	
72°C	5-10 min	

* (depends on the insert size and PCR enzymes)

Analyzing Positive Clones by restriction enzyme digestion

Inoculate positive clones on LB/Amp$^+$ or LB/Kan$^+$ liquid medium, grow at 37°C for 6 hours at 200 rpm. Isolate plasmid DNA by plasmid MiniPrep Kit. Analyze plasmid by restriction enzyme digestion with proper restriction endonuclease.

Sequencing

Analyze the sequence by sequencing with M13 F, M13 R and T7 promoter.

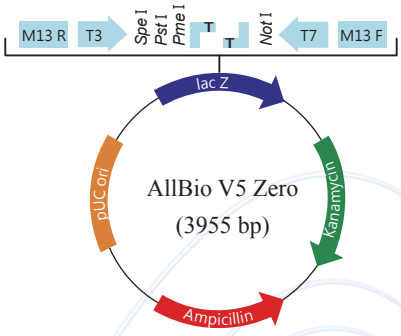
PCR for control insert (700 bp)

Component	Volume	Final Concentration
Control Template	1 µl	0.1 ng/µl
Control Primers (10 µM)	1 µl	0.2 µM
P Easy Taq PCR SuperMix	25 µl	1X
ddH_2O	Variable	-
Total volume	50 µl	-

Thermal cycling conditions for control insert

94°C	2~5min	
94°C	30 sec	30 cycles
50-60°C	30 sec	
72°C	1 kb/min	
72°C	10 min	

AllBio V5 Zero Cloning Kit



LacZα fragment: bases 217-809
M13 reverse priming site: bases 207-223
T7 promoter priming site: bases 327-346
M13 Forward priming site: bases 353-369
Kanamycin resistance ORF: bases 1158-1952
Ampicillin resistance ORF (c): bases 2202-3062
pUC origin: bases 3160-3833
(c) = complementary strand

M13 Reverse Primer

T3 Promoter Spe I Pst I Pme I

TTC ACA CAG GAA ACA GCT ATG ACC ATG ATT ACG CCA AGC TCA GAA TTA ACC CTC ACT AAA GGG ACT AGT CCT GCA GGT TTA AAC
AAG TGT GTC CTT TGT CGA TAC TGG TAC TAA TGC GGT TCG AGT CTT AAT TGG GAG TGA TTT CCC TGA TCA GGA CGT CCA AAT TTG

Not I T7 Promoter

GAA TTG GCC CTT AA GGG CCA ATT CGC GGC CGC TAA ATT CAA TTC GCC CTA TAG TGA GTC GTA TTA CAA TTC
CTT AAC CGG GAA TT CCC GGT TAA GCG CCG GCG ATT TAA GTT AAG CGG GAT ATC ACT CAG CAT AAT GTT AAG

PCR Product

M13 Forward Primer

A CTG GCC GTC GTT TTA CAA
T GAC CGG CAG CAA AAT GTT